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Effectiveness of EGFR-TKI rechallenge immediately after PD-1 blockade failure

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Abstract

Background: There is currently insufficient information available on effective therapies that can be administered to patients with non-small cell cancer (NSCLC) who develop resistance to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). However, sequential treatment via programmed death-1 (PD-1) blockade followed by EGFR-TKI rechallenge is suggested to improve the therapeutic efficacy in such patients.

Methods: A total of 75 patients with advanced NSCLC harboring sensitive EGFR mutations treated with afatinib, erlotinib, or gefitinib after EGFR-TKI treatment failure were retrospectively analyzed. Among them, 13 patients were treated with EGFR-TKI rechallenge immediately after the failure of PD-1 blockade therapy (experimental group) and the remaining 62 patients did not receive PD-1 inhibitor therapy before EGFR-TKI rechallenge (control group). Blood samples were collected at two time points; before the initiation of anti-PD-1 therapy and at EGFR-TKI rechallenge.

Results: The objective response rates of EGFR-TKI rechallenge in the experimental and control groups were 46.1% and 16.1%, respectively, with a significant difference ($p = 0.026$). In the experimental group, the median progression-free survival (PFS) and overall survival (OS) after EGFR-TKI rechallenge were 5.0 and 25.0 months, respectively, and no statistically significant difference in the percentage of lymphocytes before immune checkpoint inhibitor (ICI) therapy and EGFR-TKIs was observed in patients with partial response (PR) and without PR after EGFR-TKI rechallenge. In particular, the sequential treatment of PD-1 blockade therapy followed by EGFR-TKI rechallenge was consecutively repeated three times in two out of 13 patients in the experimental group, and EGFR-TKI rechallenge consecutively for the third time yielded a PR without increased toxicities.

Conclusions: EGFR-TKI rechallenge immediately after PD-1 blockade treatment was identified as an effective therapy for NSCLC patients with resistance to EGFR-TKIs.

KEYWORDS
EGFR-TKI, immune checkpoint inhibitor, lung cancer, PD-1, rechallenge

INTRODUCTION

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are known to be effective in patients with advanced non-small cell lung cancer (NSCLC) harboring *EGFR* mutations.[1] Three generations of EGFR-TKIs are available, which are gefitinib or erlotinib as first-generation agents, afatinib or dacomitinib as second-generation agents,

and osimertinib as a third-generation TKI. However, *EGFR* mutation is an opposite factor for the favorable prediction of the response to immune checkpoint inhibitors (ICIs), such as anti-programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) antibodies.[2] Moreover, according to recent reports, a combination of osimertinib, durvalumab, and osimertinib immediately after nivolumab therapy is not acceptable due to the increased frequency of interstitial lung disease in patients with NSCLC harboring *EGFR* mutations.[3,4] Currently, clinicians consider the usefulness of PD-1 blockade as a weak strategy for the prolongation of survival in patients with *EGFR* mutation-positive NSCLC. Notably, we have previously reported a drastic response to EGFR-TKI rechallenge immediately after PD-1 blockade in two patients with TKI-resistant *EGFR* mutation-positive NSCLC.[5] It remains unknown why EGFR-TKI rechallenge immediately after the failure of PD-1 blockade could overcome its resistance.

Recently, there have been several reports on the therapeutic efficacy of EGFR-TKI rechallenge in *EGFR*-mutant NSCLC.[6–8] Yamaguchi et al. reported that afatinib rechallenge after the failure of first-generation EGFR-TKIs exhibited an objective response rate (ORR) of 17.0% and disease control rate (DCR) of 79.2% and suggested afatinib rechallenge as one of the preferred therapeutic options in such cases.[6] In addition, first-generation EGFR-TKI rechallenge has been identified to have an ORR in patients of approximately 10%.[7,8] Its rechallenge after EGFR-TKI failure gives only limited efficacy and may slightly contribute to the prolongation of survival. There are no established treatment options for patients showing resistance after EGFR-TKI failure; thus, further research is warranted to overcome its resistance aside from the T790M mutation.

Recently, Offin et al. reported that the tumor mutational burden (TMB) on pre-EGFR-TKI was significantly lower than that after EGFR-TKI resistance, and a significantly shorter survival was associated with patients having *EGFR* mutation with a high TMB than those with a low TMB.[9] An analysis using paired pretreatment and post-progression samples revealed that TMB was significantly increased at resistance.[9] Their study suggests a close relationship between EGFR-TKI resistance and increased TMB. TMB is considered a favorable predictor of response to PD-1 blockade therapy.[10] Although NSCLC patients with *EGFR* mutations are suggested to have an increased TMB after resistance to EGFR-TKIs, it remains unclear whether PD-1 blockade is liable to suppress tumor growth. A recent study showed that the estimated five-year overall survival (OS) rate was 16% for patients receiving nivolumab monotherapy.[11] *EGFR* mutation was observed in fewer patients with a five-year survival rate, thus, PD-1 blockade provides the possibility to contribute to the long-term survival of patients with *EGFR* mutation-positive NSCLC.

Based on this background, we investigated the effectiveness of EGFR-TKI rechallenge after EGFR-TKI failure and elucidated the possibility of PD-1 blockade therapy followed by EGFR-TKI rechallenge. Moreover, we evaluated the correlation between the transition of plasma lymphocytes and the treatment course.

METHODS

Patients

From May 2014 to December 2019, 83 patients with advanced NSCLC harboring sensitive *EGFR* mutations were treated with afatinib, erlotinib, or gefitinib after EGFR-TKI treatment failure at the Saitama Medical University International Medical Center. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institution and/or the National Research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the Institutional Ethics Committee of the Saitama Medical University International Medical Center. In our retrospective study, patients who had received osimertinib immediately after the failure of PD-1 blockade therapy were excluded, and the therapeutic analysis of osimertinib as the rechallenge or in EGFR T790M-positive NSCLC was not performed. Among 83 patients, eight without evaluable lesions were excluded and the remaining 75 were eligible to be included in the analysis. In the experimental group, 13 of 75 patients who received EGFR-TKI rechallenge immediately after the failure of PD-1 blockade therapy were chosen, and the remaining 62 patients who did not receive PD-1 inhibitor before EGFR-TKI rechallenge were selected as the control group. This control group overlapped with the previously reported cases.[6] Figure 1(a) shows the CONSORT flow diagram for patient selection.

Treatment and evaluation

Afatinib, erlotinib, or gefitinib were orally administered daily, and the starting dose of these EGFR-TKIs was chosen by the chief physician. In addition, erlotinib plus bevacizumab was selected based on the judgment of the chief physician, and bevacizumab was intravenously administered at a dose of 15 mg/kg every three weeks. As PD-1 blockade monotherapy, nivolumab and pembrolizumab were intravenously administered at a dose of 240 mg/day (or 3 mg/kg) and 200 mg/day, respectively. The treatment schedule of the experimental and control groups is shown in Figure 1(b). Complete blood cell count, differential count, routine biochemistry measurements, physical examination, and toxicity levels were evaluated. Acute toxicity was graded according to the Common Terminology Criteria for Adverse Events v4.0. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors v1.1 (RECIST).[12]

Blood sample analysis

The patients in the experimental group ($n = 13$) participated in the observational study on peripheral blood mononuclear

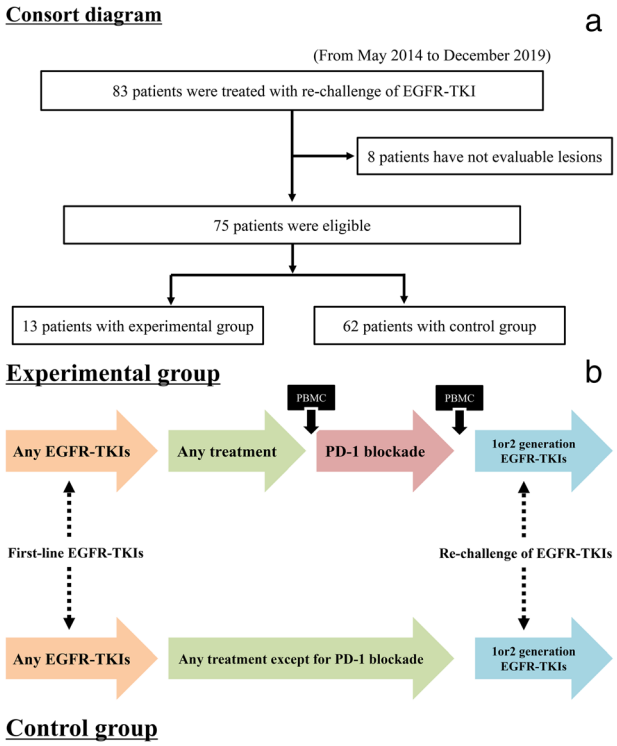

FIGURE 1 (a) Consort diagram and (b) schema of therapeutic sequence and timing of PBMC in experimental group and control group. Orange arrow, first-line EGFR-TKI; green arrow, any therapeutic chemotherapy including second or more line EGFR-TKI without PD-1 blockade; red arrow, PD-1 blockade; blue arrow, EGFR-TKI rechallenge after PD-1 blockade treatment

cell (PBMC) analysis as a predictor of PD-1 blockade. Written informed consent was obtained from all 13 patients included for the analysis of PBMC. Blood samples were collected at two time points before the initiation of PD-1 blockade therapy and EGFR-TKI rechallenge (Figure 2(b)). PBMC analysis was performed according to our previous study.[13] Cells were stained with monoclonal antibodies (mAbs) using the BD Accuri C6 plus flow cytometer (Becton Dickinson and Company) and the BD LSR Fortessa (Becton, Dickinson and Company). The following mAbs were used in the study: fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (HIT3a) and anti-CD4 (RPA-T4), phycoerythrin (PE)-conjugated anti-CD8 (RPAT8) and anti-CD25 (M-A251), PE-Cy7-conjugated anti-CD25 (MA251), PE-Cy5-conjugated anti-CD62L (Dreg 56; all from BD Pharmingen), and FITC-conjugated anti-CD62L (Dreg 56; eBioscience).

Statistical analysis

In this study, Fisher's exact test was used to determine the association between two categorical variables. The Kaplan–Meier method was used to estimate survival as a function of time and survival differences were analyzed using the log-rank test. Progression-free survival (PFS) was defined as the time from the initial administration of the EGFR-TKI rechallenge to tumor recurrence or death from any cause, while OS was defined as the time from the initial administration of the EGFR-TKI rechallenge to death from any cause. Statistical analyses were performed using the GraphPad Prism 8 software and JMP 14.0 software (SAS Institute Inc.). $p < 0.05$ was considered statistically significant.

RESULTS

Patient demographics

Table 1 shows the patient characteristics (n = 13; n_{males} = 5, $n_{females}$ = 8; median age = 65 years; age range = 39–79 years) of the experimental group compared with the control group. Six patients had a history of smoking, and a performance status (PS) of 0–1 in 11 patients and 2–3 in two patients. All patients were histologically confirmed to have adenocarcinoma. *EGFR* mutation analysis revealed seven patients with exon 19 deletion, four with L858R (exon 21), and two with other mutations. As the first-line TKI before EGFR-TKI rechallenge, eight patients were treated with gefitinib or erlotinib, four with afatinib, and one with osimertinib. In the EGFR-TKI rechallenge, eight patients were treated with gefitinib, erlotinib, or erlotinib plus bevacizumab and five with afatinib. Table 2 shows the demographics of 13 patients who received EGFR-TKIs after ICI failure. In particular, the sequential treatment with ICIs followed by EGFR-TKIs was consecutively repeated three times in Cases 11 and 13 (Figure 2).

Next, 62 patients in the control group were compared with the experimental group (Table 1). The frequency of recurrence after surgical resection was significantly higher in the control group than in the experimental group. The frequency of the T790M mutation, the number of therapeutic regimens before the EGFR-TKI rechallenge and usefulness of cytotoxic agents between first-line and EGFR-TKI rechallenge were significantly higher in the experimental group than in the control group. The administration of afatinib as an EGFR-TKI rechallenge was more frequent in the control group, and the ORR of the EGFR-TKI rechallenge was higher in the experimental group.

There were no serious side effects to EGFR-TKIs with more than grade 3 adverse events in the patients who did and did not receive ICIs.

Efficacy and survival benefit with EGFR-TKI rechallenge (experimental group)

Figure 3(a) shows the objective responses to the EGFR-TKI rechallenge after PD-1 blockade treatment. The ORR and DCR were 46.1% and 76.1%, respectively. Six of 13 patients yielded a partial response (PR), four had stable disease (SD), and three had progressive disease (PD). The ORRs of EGFR-TKI rechallenge in the experimental and control

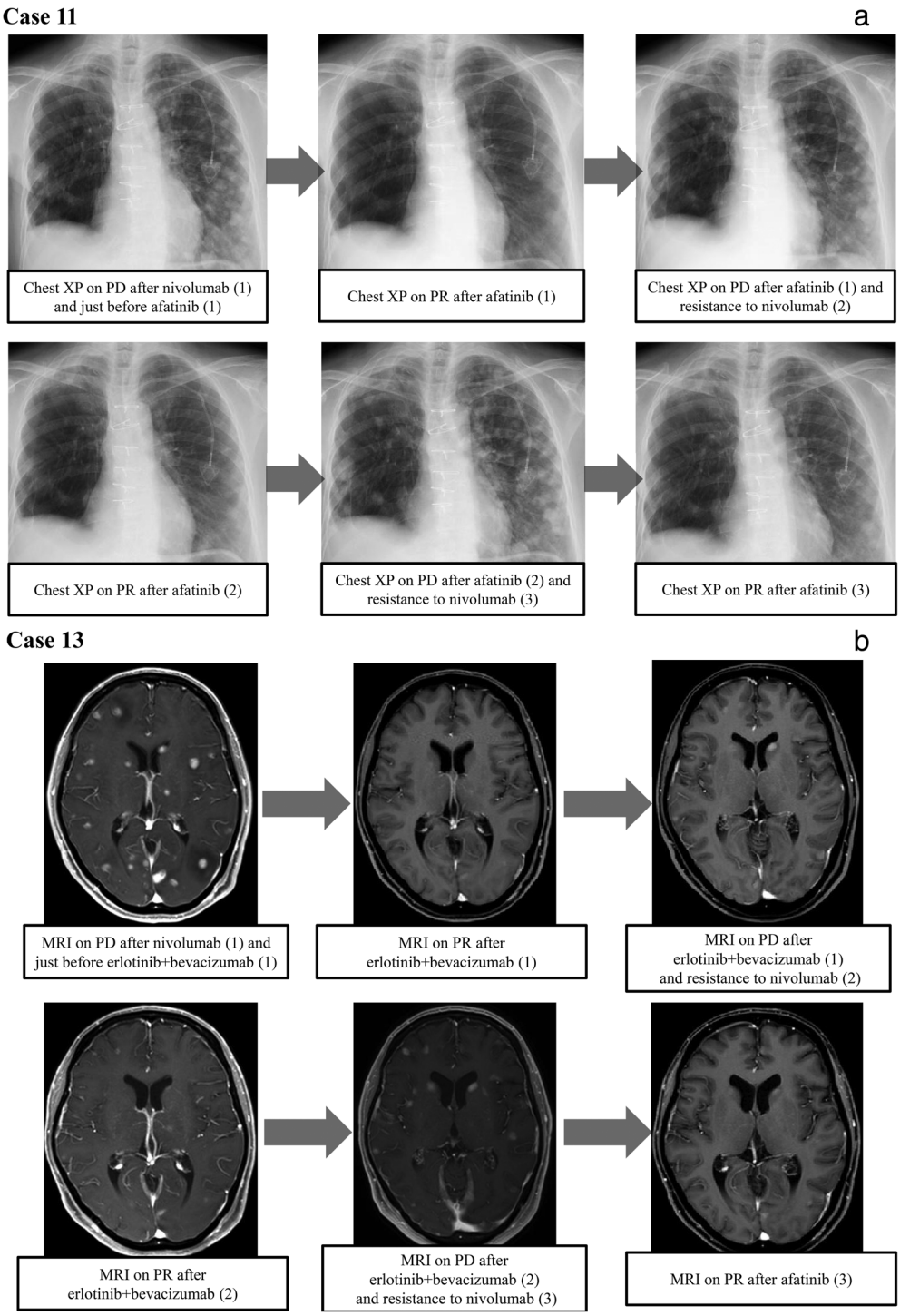

FIGURE 2 Therapeutic sequencing treatment and radiographic transition in (a) Case 11 and (b) Case 13. (a) Case 11: Chest radiograph showing therapeutic sequence after nivolumab initiation. Nivolumab was initiated, and afatinib was immediately administered after progressive disease (PD). The sequence of nivolumab followed by afatinib was repeated three times for 26 months, and the objective response rates (ORRs) of nivolumab and afatinib were PD and partial response (PR), respectively, each time. Nivolumab (1); first nivolumab, nivolumab (2); second nivolumab, nivolumab (3); third nivolumab, afatinib (1); first afatinib rechallenge; afatinib (2); second afatinib rechallenge and afatinib (3); afatinib rechallenge. (b) Case 13: Brain MRI showing therapeutic sequence after nivolumab initiation. Nivolumab was started. Similar to above Case 11, the sequence of nivolumab followed by erlotinib plus bevacizumab or afatinib was repeated three times for 28 months, and the ORRs of nivolumab and erlotinib plus bevacizumab or afatinib were PD and PR, respectively, each time. Nivolumab (1); first nivolumab, nivolumab (2); second nivolumab, nivolumab (3); third nivolumab, erlotinib plus bevacizumab (1); first erlotinib plus bevacizumab rechallenge; erlotinib plus bevacizumab (2); second erlotinib plus bevacizumab rechallenge and afatinib (3); afatinib rechallenge

groups were 46.1% and 16.1%, respectively, with a significant difference (p = 0.026). Of all patients who received PD-1 blockade monotherapy, four patients had SD and nine had PD, indicating an ORR of 0% and a DCR of 30.7%. In the experimental group, the ORR and DCR of the first-line EGFR-TKIs were 84.6% and 92.3%, respectively.

Figure 3(b) shows the total therapeutic sequence and the treatment period from the initiation of PD-1 blockade therapy is shown in Figure 3(c). PD-1 blockade monotherapy, consisting of pembrolizumab and nivolumab, was administered to four and nine patients, respectively.

Next, we analyzed the survival data of the experimental group. The median PFS of patients who received PD-1 blockade therapy was two months (range, 1.0–9.5 months), and the median PFS and OS of patients treated with the EGFR-TKI rechallenge were five and 25 months, respectively (Figure A1, online only).

TABLE 1 Comparison of patients who received EGFR-TKIs after ICIs with the control group

Variables	EGFR-TKIs after ICIs (n = 13)	Control group (n = 62)	p-value
Age			
<70 years / ≧70 years	9 / 4	31 / 31	0.237
Gender			
Male / female	5 / 8	24 / 38	>0.999
ECOG PS			
0–1 / 2–3	11 / 2	45 / 17	0.495
Smoking			
Yes / No	6 / 7	26 / 36	>0.999
EGFR mutation status			
Del 19 / L858R / other	7 / 4 / 2	37 / 18 / 7	>0.999
T790M confirmation			
Yes / No	6 / 7	10 / 52	**0.026**
Disease stage			
Stage IV/recurrence after surgical resection	12/1	44/18	**0.04**
First-line EGFR-TKIs			
Gef or Erl / Afa / Osi	8 / 4 / 1	53 / 7 / 2	0.07
Drugs used in EGFR TKI rechallenge			
Gef, Erl or Erl + Bev / Afa	8 / 5	8 / 54	**<0.001**
Response to EGFR TKI rechallenge			
PR / SD / PD	6 / 4 / 3	10 / 37 / 15	
Objective response rate	46.1%	16.1%	**0.026**
Number of therapeutic regimens before EGFR-TKI rechallenge			
1 / 2 / more 3	0 / 2 / 11	13 / 29 / 20	**0.001**
Usefulness of cytotoxic agents between first-line and TKI rechallenge			
Yes / No	13 / 0	38 / 24	**0.006**

Note: Bold character means statistical significance.

Abbreviations: Afa, afatinib; Bev, bevacizumab; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; Erl, erlotinib; Gef, gefitinib; ICIs, immune checkpoint inhibitors; Osi, osimertinib; PR, partial response; SD, stable disease.

Analysis of PBMCs in the experimental group

The analysis of peripheral blood mononuclear cells (PBMCs) was successfully performed in 11 of 13 patients at two time points, that is before the ICI and EGFR-TKI treatments; however, PBMCs were collected only before ICI therapy without EGFR-TKIs in the remaining two patients. In particular, blood samples at two time points, that is before the ICI and EGFR-TKI treatments, were collected three times in Cases 11 and 13. Figure 3 shows the transition of CD4, CD8, Foxp3, and $CD62L^{low}$ before the ICI and EGFR-TKI treatments. No statistically significant differences in the percentage of these lymphocytes were observed before the ICI and EGFR-TKI treatments in patients with PR (Figure 4(a)) and without PR (Figure 4(b)) after the EGFR-TKI rechallenge.

Dramatic response by EGFR-TKI rechallenge in two cases

As described above, Case 11 and Case 13 repeatedly received the sequential ICI regimens followed by EGFR-TKIs (Figure 2), as previously reported.[5]

Case 11 A 64-year old woman with adenocarcinoma repeatedly received sequential treatments, including EGFR-TKI and cytotoxic agents (Figure 2(a)). As there was a resistance to the EGFR-TKI and systemic chemotherapy, nivolumab was initiated. One month after its administration, PD was observed; therefore, the patient was treated with afatinib rechallenge. Two weeks after treatment, a drastic response of tumor shrinkage in multiple pulmonary metastases was identified. Ten months after the rechallenge, pulmonary metastases exhibited marked regrowth, and nivolumab was restarted. Because of immediate tumor progression, afatinib rechallenge was repeated for a second time. The tumor regression continued for seven months, and the patient also had tumor regrowth of pulmonary metastases. Nivolumab as a rechallenge was administered for the third time; however, progression of pulmonary metastases also occurred. Considering the possibility of nivolumab followed by afatinib therapy, afatinib as a rechallenge was administered for the third time and the patient had tumor regression and continued to receive treatment.

TABLE 2 Patient demographics of 13 patients who received EGFR-TKIs after ICI failure

								ICI		EGFR-TKIs after ICIs		
Case	**Age**	**Gender**	**PS**	**Smoking**	**TNM**	***EGFR* mutation**	**Therapeutic sequences before ICIs**	**Drug**	**OR**	**Drug**	**OR**	**Gr3 or 4 AE / ILD**
1	62	Male	0	Yes	T2aN3M1c	L858R	Afa → Osi → CDDP/DTX	Pem	SD	Afa	PD	None / none
2	66	Female	0	No	T4N3M1a	L858R	Erl → CDDP/PEM/Bev → Erl → Osi	Pem	SD	Afa	PD	None / none
3	46	Female	3	Yes	T4N3M1c	Del 19	Gef → Erl → Afa → Osi → Erl/Bev → PEM/Bev	Nivo	PD	Afa	PD	None / none
4	71	Male	2	Yes	T2aN3M1b	Del 19	Erl → Osi → CBDCA/nabPTX→PEM	Nivo	SD	Erl/Bev	SD	None / none
5	65	Female	1	No	T1cN3M1c	Del 19	Afa → CBDCA/PEM/Osi	Nivo	PD	Erl/Bev	SD	None / none
6	79	Male	1	Yes	Ope rec.	Del 19	Gef → CDDP/PEM → Gef → PEM → Gef → Afa→ DTX/S-1	Nivo	SD	Erl	SD	None / none
7	77	Female	1	Yes	T1bN0M1b	Del 19	Gef → CBDCA/PEM → Afa → DTX → Osi	Nivo	SD	Erl/Bev	SD	None / none
8	72	Male	1	No	T2aN3M1b	L858R	Erl → CBDCA/PEM/Bev → Erl → Osi	Nivo	PD	Erl/Bev	PR	None / none
9	67	Female	1	Yes	T2bN3M1b	Del 19	Afa → CDDP/VP-16	Nivo	PD	Afa	PR	None / none
10	65	Female	1	No	T3N0M1c	L858R	Osi → CBDCA/PEM → Erl/Bev	Pem	PD	Erl/Bev	PR	None / none
11	64	Female	0	No	T4N3M1a	L851Q	Gef → CBDCA/PEM/Bev → Erl → DTX → Afa→ CBDCA/PEM → Erl/Bev	Nivo	PD	Afa	PR	None / none
12	40	Male	0	Yes	T2aN3M1b	Del 19	Erl → CBDCA/PEM/Bev	Pem	SD	Erl/Bev	PR	None / none
13	39	Female	1	No	T2bN3M1c	Del 19	Afa → Osi → CDDP/PEM/Bev → Osi	Nivo	PD	Erl/Bev	PR	None / none

Abbreviations: AE, adverse events; Afa, afatinib; Bev, bevacizumab; CBDCA, carboplatin; CDDP, cisplatin; Del 19, deletion 19; DTX, docetaxel; EGFR mutation, epidermal growth factor receptor mutation status; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; Erl, erlotinib; Gef, gefitinib; ICI, immune checkpoint inhibitor; ILD, interstitial lung disease.; nabPTX, nab-paclitaxel; Nivo, nivolumab; Ope rec., recurrence after operation; OR, objective response; Osi, osimertinib; PD, progressive disease; Pem, pembrolizumab; PEM, pemetrexed; PR, partial response; PS, performance status; RT, radiation; SD, stable disease; VP-16, etopiside.

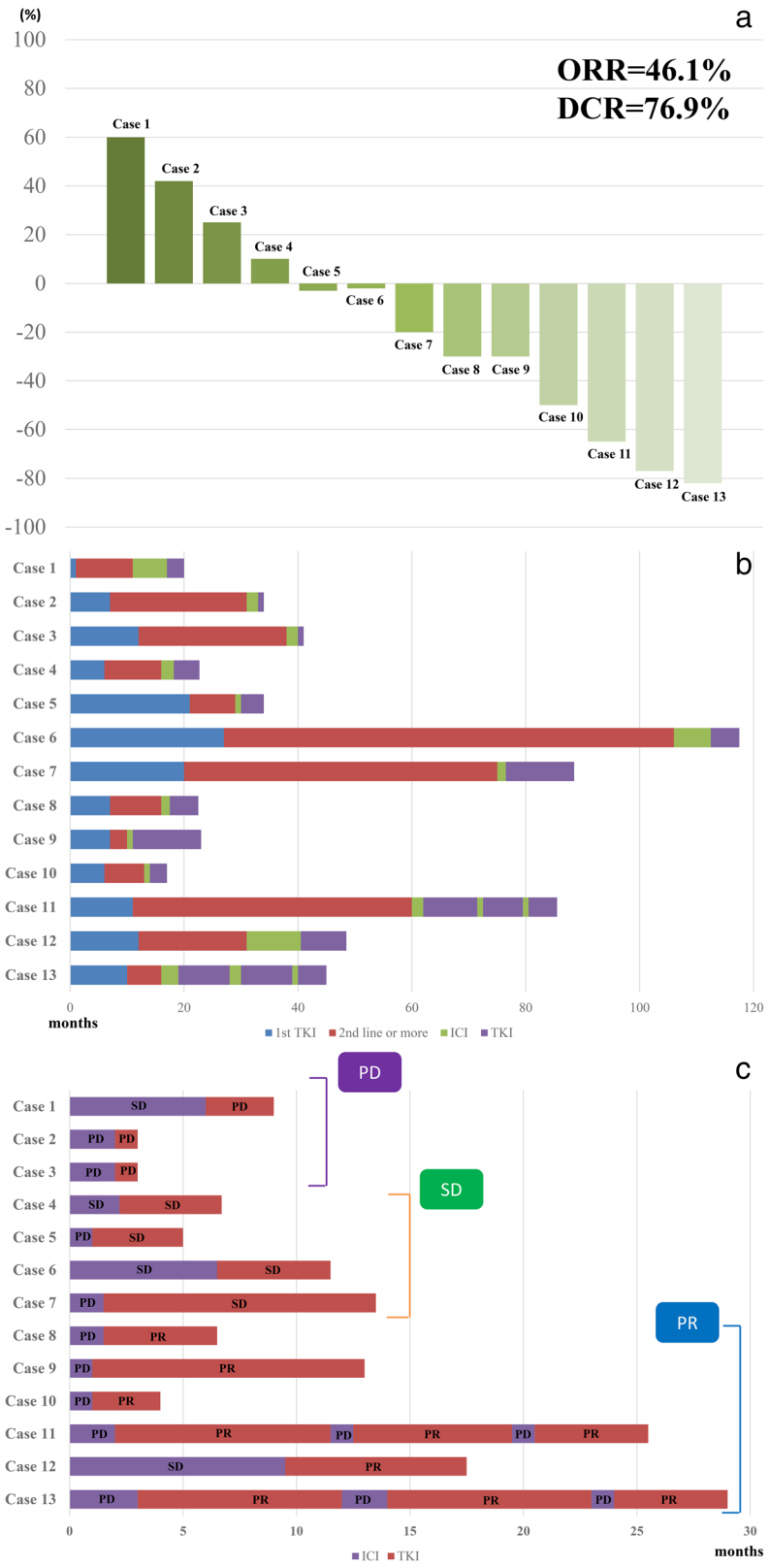

FIGURE 3 Waterfall plots in objective response on EGFR-TKI immediately after PD-1 blockade treatment in the experimental group (a). Objective response rate (ORR) was 46.1% and disease control rate was 76.9%. Swimmer's plots of therapeutic sequencing treatment from initial EGFR-TKI to last follow-up date (b). Blue bar, the therapeutic period of first line EGFR-TKI; red bar, from second line or more lines just before PD-1 blockade; green bar, treatment period of PD-1 blockade; purple bar, EGFR-TKI rechallenge. Swimmer's plots of treatment period from PD-1 blockade initiation to last follow-up date (b). The therapeutic periods of PD-1 blockade and EGFR-TKI rechallenge are shown by purple and red bars, respectively. Cases 1, 2 and 3 experienced progressive disease (PD) to EGFR-TKI rechallenge, Cases 4, 5, 6 and 7 maintained stable disease (SD) and Cases 8, 9, 10, 11, 12 and 13 had a partial response (PR)

Case 13 A 39-year old woman with adenocarcinoma was repeatedly treated with second- or third-generation EGFR-TKI or platinum-based regimens; however, the patient experienced therapeutic resistance (Figure 2 (b)). Thus, nivolumab was initiated, but there was evidence of marked expansion of multiple brain metastases one month after its administration. Erlotinib plus bevacizumab was sequentially administered, and the brain metastases almost disappeared after one month. Nine months after combination

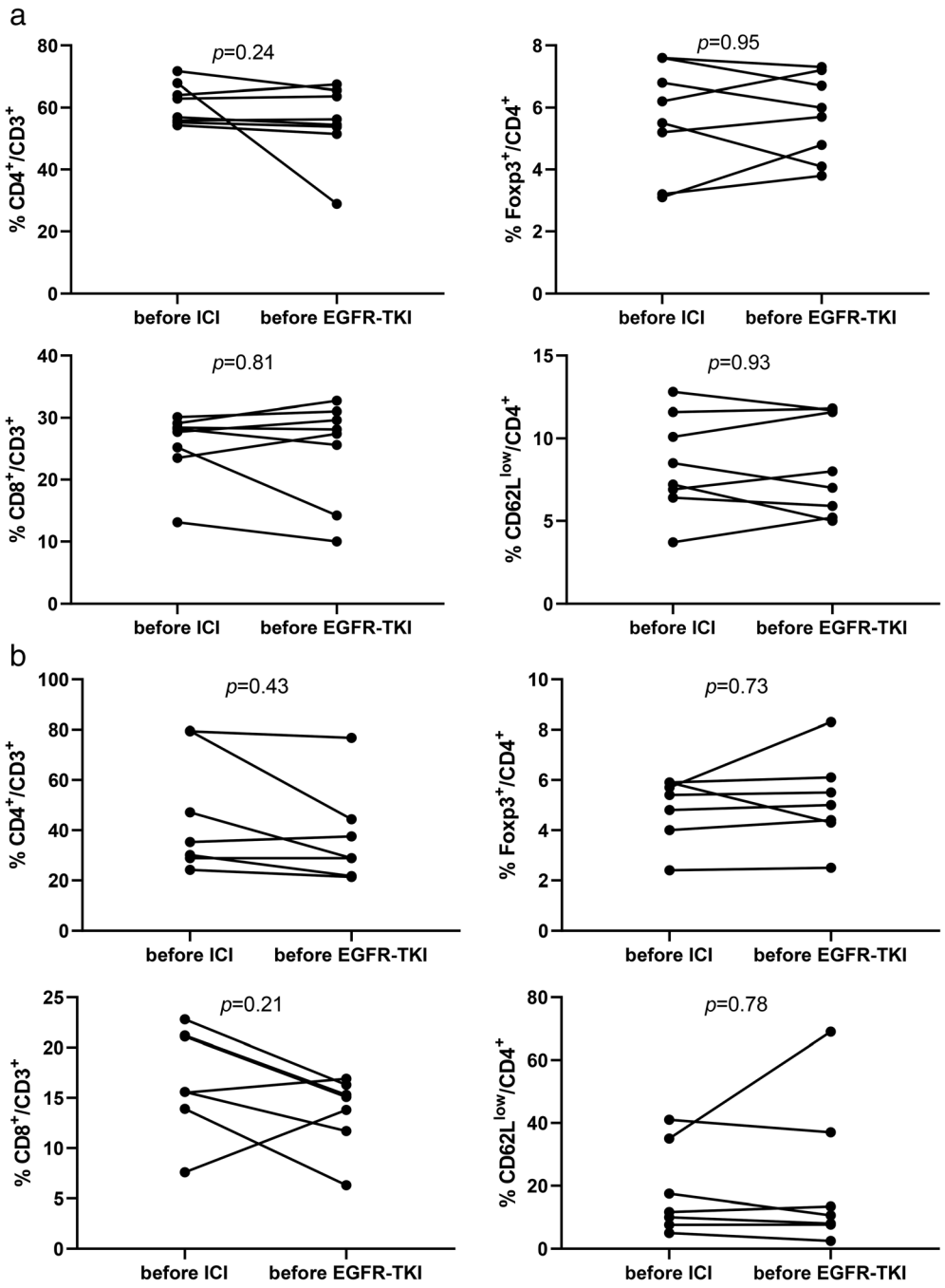

FIGURE 4 Transition of lymphocytes using PBMC analysis (CD4, CD8, Foxp3 and $CD62L^{low}$) in patients with (a) PR and (b) non-PR after EGFR-TKI rechallenge. Analysis of PBMC was performed at two points before ICI and EGFR-TKI treatment. No statistically significant difference in the percentage of these lymphocytes between before ICI and EGFR-TKI was observed in (a) PR and (b) non-PR groups

therapy, the patient experienced obvious recurrence of brain metastasis, and was treated with nivolumab rechallenge. However, exacerbation of brain metastases was identified one month after treatment. Erlotinib plus bevacizumab was started as a rechallenge for the second time, and tumor regression was observed. Five months after its rechallenge, there was slight regrowth of brain metastases. Thus, docetaxel with ramucirumab followed by osimertinib was initiated. However, the patient also experienced exacerbation of brain metastases, and nivolumab followed by afatinib was considered an effective sequential therapy. Tumor regression of brain metastases was then observed.

In Cases 11 and 13, a significant transition of lymphocytes, such as CD4, CD8, Foxp3, and $CD62L^{low}$ before the ICI and EGFR-TKI treatments was not identified (-**Figures A2** and **A3**, online only).

DISCUSSION

This is a complementary investigation to improve the efficacy of EGFR-TKI rechallenge in patients with advanced NSCLC with EGFR-TKI resistance. Although the poor response to PD-1 blockade therapy in NSCLC patients with *EGFR* mutations is consistent with a previous study,[14] the

administration of PD-1 blockade therapy just before the administration of EGFR-TKI may be helpful for the recovery of drug resistance related to the *EGFR* signaling pathway. Some patients benefited clinically from EGFR-TKI rechallenge after exacerbation of PD-1 blockade; however, the tumors of other patients failed to evade drug resistance. We observed that EGFR-TKI rechallenge immediately after PD-1 blockade could improve its therapeutic response compared to that without the previous administration of PD-1 blockade therapy. Moreover, the administration of PD-1 blockade therapy in patients with EGFR-TKI resistance did not affect the transition of lymphocytes such as CD4, CD8, and Foxp3 in the peripheral blood. However, it remains unclear why PD-1 blockade immediately before EGFR-TKI administration could change the sensitivity of tumors to EGFR-TKIs. Further studies are warranted to elucidate the detailed mechanism of the synergistic relationship between the immune microenvironment and *EGFR* driver mutations.

Little is known about the precise mechanism by which PD-1 blockade is potentially effective in patients with resistance to EGFR-TKIs. In our study, approximately half of the enrolled patients showed a good response to EGFR-TKI rechallenge, whereas there was no response to EGFR-TKI rechallenge in about 25% of all patients. In particular, Case 11 and Case 13 had long-term exposure to EGFR-TKI treatment, and their sensitivity to EGFR-TKI rechallenge repeatedly recovered after exposure to PD-1 blockade. Moreover, these two cases acquired complete resistance to PD-1 blockade, and a significant transition of lymphocytes was not identified before and after PD-1 blockade initiation. We suspect therefore that some changes in immune cells and PD-L1 expression within tumor cells may occur in the tumor immune microenvironment after PD-1 blockade administration. As it is impossible to obtain tumor specimens by biopsy, this is a matter for speculation. Recently, Sugiyama et al. reported that erlotinib as an *EGFR* inhibitor decreased CD positive effector regulatory T cell infiltration in the tumor microenvironment, but the combination of an *EGFR* inhibitor with PD-1 blockade yielded favorable antitumor effects than either treatment alone based on their experimental model.[15] Their study suggests that EGFR-TKI when administered in conjunction with PD-1 blockade potentially improve the efficacy of immunotherapy. As the mechanism of potential effect, a synergistic effect of EGFR-TKI with PD-1 blockade may recover the antitumor effect in the situation of long-term exposure to EGFR-TKI treatment.

Meanwhile, TMB might have increased compared to prior EGFR-TKIs, although TMB testing was not performed in all cases. A previous report suggested that TMB was increased in response to EGFR-TKIs[9]; therefore, a majority of our patients may have exhibited a high TMB before the initiation of the PD-1 blockade therapy. Of note, five (83.3%) of six patients with a PR to EGFR-TKIs immediately after PD-1 blockade showed a PD after PD-1 blockade therapy, whereas five (71.4%) of seven patients who showed an SD or a PD with the EGFR-TKI rechallenge yielded an SD after PD-1 blockade treatment (Table 2). The introduction of PD-1 blockade therapy may restore EGFR-TKI sensitivity in patients with drug resistant tumors. In particular, the noneffective microenvironment associated with PD-1 blockade therapy seems to encourage the therapeutic efficacy of the EGFR-TKI rechallenge. Although it is difficult to elucidate the detailed mechanism regarding the recovery of sensitivity to the EGFR-TKI rechallenge immediately after PD-1 antibody therapy, further investigations on such promising subsequent therapies should be considered.

Recently, Isomoto et al. demonstrated the immune microenvironment in tumor rebiopsy samples after progression during the EGFR-TKI treatment.[16] In their investigation, the expression level of PD-L1 increased, TMB tended to be higher, and CD8 and Foxp3 tumor-infiltrating lymphocytes were significantly lower after EGFR-TKI treatment than before treatment.[16] Therefore, the efficacy of the PD-1 blockade therapy is suggested to be dependent on the expression level of PD-L1 after EGFR-TKI treatment.

In the present study, we explored the transition of lymphocytes in the systemic peripheral blood at two time points before PD-1 blockade therapy and EGFR-TKI rechallenge using PBMC analysis. The results of our study indicated that the initiation of PD-1 blockade therapy did not have an impact on the movement of peripheral lymphocytes. As we could not determine the dynamic transition of the immune microenvironment apart from the peripheral blood, it remains unknown how immune cells change within tumor specimens. However, the case presentations with regard to Cases 11 and 13 revealed interesting findings. The sequential treatment of PD-1 blockade followed by EGFR-TKI rechallenge was repeated in the same patient; however, this sequence was consistently effective, suggesting the recovery of sensitivity to EGFR-TKI. To our knowledge, this is the first study to show an improved tolerance. If dynamic monitoring of the immune microenvironment in tumor specimens is possible, we may be able to discover new evidence on therapeutic responses. In fact, dynamic monitoring of tumor tissue is actually impossible; thus, it may be difficult to elucidate the detailed mechanisms underlying the present findings. Considering the paradoxical phenomenon in our cases, we hypothesize that the effect of cancer immunoediting by PD-1 blockade therapy before EGFR-TKI administration encourages driving out the resistant tumor cells with additional gene mutations, then EGFR signal-dependent cancer cells are dominantly close to the original one without additional mutations. Therefore, the therapeutic response of EGFR-TKIs immediately after PD-1 blockade therapy may be similar to that of first-line EGFR-TKIs. However, little is known about the detailed mechanism that can be used to elucidate the phenomenon involved in our study.

There are several limitations to our study. First, the present study involved a small sample size assessed using a retrospective approach; thus, it may have contributed to biased results. Further confirmation of the therapeutic significance of PD-1 blockade therapy followed by EGFR-TKIs is warranted in a prospective large-scale study. Second, a

comprehensive analysis of genomic alterations after resistance to EGFR-TKIs was not performed. Although the analysis of genomic alterations before and after the initiation of the PD-1 blockade therapy is an interesting issue, we could not obtain the tumor specimens for these analyses. Recently, liquid biopsy has been described to be available for the detection of genomic alterations using next-generation sequencing (NGS) analysis, but this procedure appears to yield several limitations. Finally, the EGFR-TKIs administered in our study were not uniform; thus, it remains unclear which generation of EGFR-TKIs, such as first- or second-generation are most effective as a sequential therapy after PD-1 blockade therapy. A prospective study is therefore required to resolve this limitation.

In conclusion, EGFR-TKI rechallenge immediately after PD-1 blockade treatment was identified as an effective therapy for NSCLC patients with resistance to EGFR-TKIs. Although the reason for this finding is unclear, EGFR-TKI rechallenge may be effective for patients with PD after PD-1 blockade as a prior treatment. Physicians should therefore be alerted to the choice of PD-1 blockade therapy followed by EGFR-TKI rechallenge as a clinical practice.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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